CATARRHAL AND PURULENT CONJUNCTIVITIS IN THE NEW ZEALAND WHITE AND CALIFORNIAN RABBITS

by

GEORGE EDWIN KING

B. S., Kansas State College
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D. V. M., Kansas State College
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INTRODUCTION

Conjunctivitis is a serious and economic problem in rabbitries. It affects both commercial and show animals. Conjunctivitis is defined as inflammation of the conjunctiva, according to Dorland (27).

The condition is also referred to as watery eyes, weeping eyes or running (pus) eyes. The rabbit breeder and commercial raiser are interested in the prevention and the control of conjunctivitis.

Rabbits that are affected are discriminated against in the show ring and are not accepted on the commercial market. This is because of the unsightly condition of the face, manifested by its wet fur or the denuded area that occurs after the condition has progressed for a period of ten days or more. There is a chronic discharge from the eye which may be thin or thick in character. The color may be clear to yellow or white. One or both eyes may be affected.

The condition progresses rather slowly over a period of a few weeks and then it may recede gradually without treatment. If recovery occurs, the hair begins to grow, the exudate on the face and in the conjunctival sac disappears and the rabbit has apparently recovered.

The available literature on conjunctivitis in rabbits is definitely limited. Because of this limitation and the importance of the rabbit industry, the disease of conjunctivitis in rabbits warrants investigation.

REVIEW OF LITERATURE

Lund (49) lists the etiology of conjunctivitis as irritation, usually followed by or accompanied with infection with any of several common bacteria. He states that one animal in the litter is often a carrier of infection to the other animals.

Perera (62) has listed the varieties of conjunctivitis in man as follows:

1. Catarrhal: (a) acute, (b) chronic.
2. Follicular: (a) acute, (b) chronic.
3. Purulent: (a) ophthalmia neonatorum, (b) gonorrheal.
4. Membranous: (a) diphtheritic, (b) non-diphtheritic.
5. Trachoma and inclusion conjunctivitis.
6. Phlyctenular.
7. Allergic and Vernal.

Blennorrhagic ophthalmia is mentioned by the older French veterinary authors, meaning ophthalmia with a discharge of purulent exudate, but in man it has a more definite meaning, in which case it means infection by the pathogenic organism in the purulent exudate of gonorrhea or blennorrhagia (60).

In chickens, conjunctivitis may be caused by the nematode, Oxyspirura mansoni. Morgan and Hawkins (55) state that the symptoms of lacrimation, nasal discharge and occasionally opacity of the cornea may be seen after the nematodes have disappeared from under the nictitating membrane. According to these authors, the severity of the symptoms may increase

due to the irritation and initiation by the nematodes and the secondary bacterial infection that results.

Delmer, cited by Nicolas (60) transmitted acute purulent conjunctivitis in goats by taking the purulent exudate from the eyes of infected animals and instilling it on the healthy conjunctiva of other goats. The type of the bacteria in the purulent exudate was not mentioned. Nicolas (60) states that the agent or means of transmission to the eye causing purulent conjunctivitis is not known, but it would suggest some kind of infection.

In Gray's opinion, cited by Kirk (46), acute purulent conjunctivitis of the cat and the dog, the great majority of cases are a special manifestation of distemper. Kirk (46) states that there appears to be occasions, having more or less lengthy intervals, upon which there is a type of distemper principally characterized by intense chemosis and everted eyelids. The cases show a swollen conjunctival mucous membrane, which secretes a thick, creamy or yellowish purulent material. This is generally termed the "pink-eye" of distemper in the canine and feline.

Moraux as cited by Nicolas (60), has shown that a fairly virulent Streptococcus injected under the conjunctiva of rabbits causes a generalized inflammation with a well marked purulent secretion. In the human, Thygeson cited by Vail (81), has shown that a form of conjunctivitis, with fairly definite characteristics, is caused by certain strains of Staphylococci which form a soluble toxin. The strains fermenting mannite

are nearly always toxin forming, and he states that the presence of mannite fermenting colonies should be considered probable evidence that they are the cause of conjunctivitis.

Müller and Glass (56) state that in the dog, purulent conjunctivitis is due to a specific infectious organism and it is manifested during the course of some epizootic disease, such as distemper. They also mention that it is possible to produce the same form of the infection by inoculation of the conjunctiva of a healthy dog with the purulent material. They do not identify the bacteria in the purulent material. These authors mention that catarrhal conjunctivitis shows the symptoms of intense redness of the conjunctiva and, in the early stages, there is a slight increase of the flow of tears and, in later stages, a muco-purulent discharge is observed. The discharge frequently flowing down the side of the face is often noticed.

Vail (81) states that in the human, acute catarrhal conjunctivitis or "pink-eye" is caused by a pneumococcus in The United States, while in the tropical climates and the seacoast towns of Europe, the most common offender is the Koch-Weeks bacillus. Burrows (15) states that the Koch-Weeks bacillus is probably the same as Hemophilus influenzae. Dubos (30) states that the most common organism in the human eye is Corynebacterium xerosis, a harmless saprophyte which can be ignored. Perera (62) stated that the normal conjunctival sac is seldom free from microorganisms, staphylococci and

the Xerosis bacillus being present at times. Gifford (35) is in agreement with this and he states the special conditions present in the conjunctival sac make it necessary for the bacteriology to be considered. Gifford list the bacteria of the normal conjunctiva as Staphylococcus albus, Bacillus xerosis, Staphylococcus aureus, streptococci, pneumococci, Bacillus pyocyaneus, molds and higher bacteria. Thygeson, cited by Berens (11), states that for the diagnosis of the majority of the acute conjunctival diseases that a direct microscopic examination of the conjunctival material usually suffices, and that cultures are needed only for the confirmation. He also states that in the chronic forms of conjunctivitis, there are usually relatively few bacteria present and cultures have a greater value.

Lindner, cited by Gifford (35), has shown that infection of the conjunctiva occurs when large numbers of the pathogenic organisms enter the epithelial cells. The deeper layers of the epithelium are involved when still greater numbers of the pathogenic organisms are present. The toxins liberated in the deeper cells cause these cells to show the usual signs of inflammation.

Gifford (35) states that in the human, congenital stenosis of the nasolacrimal duct is much more common than is generally believed, and since it shows symptoms similar to conjunctivitis, it is often mistaken for conjunctivitis.

Law (48) states that the most prevalent symptom in stenosis

of the nasolacrimal duct in animals is the escape of tears onto the animal's face. The occlusion may be caused by the pressure from the inflammed mucosa in nasal catarrh. de Graef, cited by Law (48), stated as early as 1864, that every conjunctivitis which has a secretion is inoculable and by this virtue, it is transmissible.

The character of the inflammation of the eye varies with the type of the disease present, but the symptoms of pain, photophobia and lacrimation are common to all inflammatory diseases of the eye, and by themselves are of little value in the differential diagnosis according to Douthwaite (29). Alt (3) states that the discharge from an eye suffering from any form of conjunctivitis, except the phlyctenular, is contagious. He states, in addition, that purulent conjunctivitis is really an exaggerated catarrhal conjunctivitis and that one may pass over into the other. The symptoms of an acute purulent conjunctivitis are similar therefore to those of an acute catarrhal conjunctivitis, only more intensified and with a different character of the secretion. Van Mater (82) is of the opinion that xerophthalmia may result from a severe chronic conjunctivitis.

The term purulent conjunctivitis properly applies to all forms of conjunctivitis in which the discharge present is more or less copious and comparatively free from mucin. There are a relatively large number of forms of conjunctivitis which are mild in character and tend to recover spontaneously, without

serious complications, according to de Schweinitz and Randall (26). These authors also mention lachrymal conjunctivitis that accompanies dacryocystitis, and they state that it is due to the presence of the irritating purulent secretion from the lachrymal sac, which contains streptococci.

Berens (11) states that lysozyme, a colloidal enzyme, has been found in tears, and in the concentration found it can kill by lysis most of the common pathogenic organisms.

Watery eyes are a very common condition in the chinchilla. According to Kennedy (44), it may be caused from insanitary conditions, the starting of colds, the use of ultra-violet lamps shining on the eyes, or the tear duct becoming occluded. He also states that it may be due to a dietetic condition such as avitaminosis A. The symptoms seen are similar to those observed in rabbits. Catarrhal and purulent conjunctivitis are also found in the chinchilla. Kennedy states that ordinary infections are always present and will develop when the resistance of the chinchilla is lowered. In addition, Kennedy states that Staphylococci may be found occasionally affecting the eyes. Pseudomonas aeruginosa infection will also show conjunctivitis in the chinchilla.

In cattle, Barner (8) lists the symptoms of bovine keratitis as consisting of a conjunctivitis and lacrimation of a thin, clear watery fluid which was on occasion somewhat muco-purulent. Baldwin (7) states that in his experiments, mice, guinea pigs and rabbits were not susceptible to Hemophilus bovis.

Dukes (31) states that in vitamin A deficiency, chronic conjunctivitis is an early symptom and the condition may progress as far as xerophthalmia. Maynard (50) is not entirely in agreement with this; he states that xerophthalmia is noted particularly in avitaminosis A in children and rats, but it is not a common symptom in other animals, although corneal changes may occur. Copious lacrimation is a more prominent symptom of the eyes in cattle, during avitaminosis A. Thickening of the conjunctiva occurs with considerable frequency in groups of people that are malnourished according to Jolliffe et al. (43). When the vitamin A reserves of the body have been depleted, the animal is more susceptible to bacterial invasion, and the lowered body resistance is manifested in various ways, especially in the development of characteristic eye diseases such as ophthalmia, xerophthalmia, keratomalacia, conjunctivitis or keratoconjunctivitis according to Sherman and Smith (72). Chick and Roscoe (22) describe a marked conjunctivitis in rats on a diet deficient in riboflavin.

Blakemore (12) describes a specific conjunctivitis of sheep in England, characterized by the presence of inclusion bodies in the superficial cells of the conjunctiva. He states that the disease is not uncommon. Newsom (58) states that sheep may have conjunctivitis associated with infectious keratitis, vitamin A deficiency, pasteurellosis, sheep pox and rinderpest. Barner (8) reported a P.P.L.O. (pleuro-pneumonia-like-organism) from the eyes of sheep that were infected with infectious ovine

keratitis. Splitter (76) has made the same observation as Barner.

There is a conjunctivitis in swine infected with acute hog cholera. This is the muco-purulent type of conjunctivitis (5), (45). Craig (24) states that in hog cholera, as in all febrile disturbances, the secretion from the conjunctiva is increased, the secretion being watery at first, but soon becoming heavy and purulent. Twiehaus (80) has shown that swine infected with infectious atrophic rhinitis show a conjunctivitis and the eyes have a reddish appearance.

According to Bensley (10), the orbital opening of the nasolacrimal canal is found on the ventral side of the lacrimal bone. The opening for the nasolacrimal duct is at the junction of the lower eyelid and the membrana nictitans. The nasolacrimal canal is the osseous structure which encloses the nasolacrimal duct. In the natural condition, the nasolacrimal duct leads from the corneal surface of the eye to the anterior portion of the nasal fossa.

Erythrocyte Counts

The erythrocytes of the rabbit, like those of most mammals, are non-nucleated, biconcave discs which, upon staining with Wright's stain, appear orange in color. The average of the reported values for erythrocyte numbers in adult rabbit blood, regardless of sex or strain is 5,610,000 per cu. mm. as reported by Gardner (34). Cole et al. (23) reported that the average erythrocyte count of rabbits that were fed was 5,710,000 per

cu. mm. and by starving these rabbits for 24 hours the average erythrocyte count dropped to 5,390,000 per cu. mm. Wintrobe et al. (88) report that there is a sex difference of the average erythrocyte count. Thirty five males had an average erythrocyte count of 6,250,000 per cu. mm. and 26 females had an average count of 6,300,000 erythrocytes per cu. mm. Casey et al. (19) found that the heavier the breed of the rabbit used, the lower the erythrocyte count. Casey et al. (20) found that there is a difference in the hemogram in relation to the breed of rabbit used for the study. Sharpe and Bisgard (71) found the average erythrocyte count to be 6,150,000 per cu. mm. in the rabbits that they used. Rosahn et al. (66) reported the average erythrocyte count on 41 males to be 5,640,000 per cu. mm. while in 41 females the average erythrocyte count was 5,240,000 per cu. mm. Using 32 male rabbits, Guest, reported by Wintrobe (87), found the average erythrocyte count to be 5,890,000 per cu. mm. and with 30 females the count was 5,600,000 per cu. mm. Bushnell and Bangs (16) found the average erythrocyte count to be 6,600,000 per cu. mm. in 100 rabbits. Jackson and Stovall (42) state the average erythrocyte count of 22 rabbits studied by them was 5,346,000 per cu. mm. Although the erythrocyte count decreases during the first week of a rabbit's life, it reaches the level of the normal adult by the third week of life, according to Sabin et al. (68). Pearce and Casey (61) found the average erythrocyte count to be 5,198,000 per cu. mm. plus or minus 12,700 per cu. mm. on 174 male rabbits of various ages and breeds.

Mole (52) found the average erythrocyte count of 19 rabbits as 6,567,609 per cu. mm.

Hemoglobin

The average of the reported amounts of hemoglobin in the blood of adult rabbits is 12.07 grams per 100 cc., regardless of the sex or strain of the animal, as reported by Gardner (34). Sharpe and Bisgard (71), using the Sahli method, reported finding 14.7 grams per 100 cc. Wintrobe et al. (88) found 12.9 grams per 100 cc. in 35 males and 13.4 grams per 100 cc. in 26 females. They used the Newcomer method. Guest (Wintrobe, 87) found 12.9 grams per 100 cc. in 32 males and 13.0 grams per 100 cc. in 30 females. Casey et al. (19) found that the heavier breeds of rabbits had a significantly lower hemoglobin content than the smaller breeds of rabbits. Rosahn et al. (66) state that 41 male rabbits had a higher hemoglobin content than their litter mate sisters and the difference was due to constitutional differences between the two sexes.

Hematocrit

As reported by Gardner (34), the average of the reported values for volume of packed red cells in adult rabbits is 41.39 per cent, regardless of the sex or strain of the animal. Cole et al. (23) found that a starvation period of 24 hours lowered the packed red cells from 42.2 per cent to 38.6 per cent. Wintrobe et al. (88) reported a sex difference in the packed red

cell volume, 35 males had an average of 39.4 per cent while 26 females had an average of 40.1 per cent. Sharpe and Bisgard (71) reported the packed red cell volume as 38.8 per cent. Guest (Wintrobe, 87) reports the packed red cell volume of 32 male rabbits averaged 38.6 per cent. Rapoport et al. (64) report the average packed red cell volume of five New Zealand White rabbits as 41.8 per cent, with a range of 37.2 per cent to 46.6 per cent. Hueper et al. (40) states that the average packed red cell volume is 46 per cent.

Sedimentation Rate

Gregg (37) found the sedimentation rate of erythrocytes in rabbit blood to be within a range of 2.0 mm. to 4.0 mm. in three hours. Sharpe and Bisgard (71) found the sedimentation rate of erythrocytes of the blood of normal rabbits to average 1.0 mm. per hour. Hueper et al. (40) found no sedimentation at the end of one hour in normal rabbit blood.

Dougherty and White (28) determined the specific gravity of whole blood of rabbits by the falling drop method of Kagan (Wintrobe, 87). They report an average specific gravity of 1.053 plus or minus 0.0009.

Leukocyte and Differential Leukocyte Counts

The averages of the reported values for the different types of leukocytes, expressed in per cent, as reported by Gardner (34) are as follows:

Pseudo-eosinophils (neutrophils)	45.03 (31.7-59.3)
Eosinophils	1.62 (0.7-4.3)
Basophils	6.28 (2.4-9.0)
Monocytes (large mononuclears)	9.54 (1.3-15.8)
Lymphocytes (small mononuclears)	38.45 (19.8-68.2)

The leukocytes occurring in the greatest numbers in the normal adult rabbit is the pseudo-eosinophil which is analogous to the neutrophil of human blood, according to Gardner (34). The eosinophil granules are three to four times larger than the pseudo-eosinophil granules and usually cover the cell, while the granules of the pseudo-eosinophil are smaller and of uniform size. Pearce and Casey (61) report that on 174 rabbits of various ages and breeds they found the leukocyte count to average 9,562 per cu. mm. Cole et al. (23) found that by starving 28 rabbits for 24 hours the leukocyte count dropped from 8,900 per cu. mm. to 8,340 per cu. mm. Nice and Katz (59) found a 14.3 per cent change in the leukocytes, which was a delayed leukopenia, after excitement in 20 rabbits that they tested. Casey (18) made 963 total white cell counts on 204 young adult male rabbits between the hours of 9 a.m. and 5 p.m. and they revealed no statistically significant variation in the hourly means, nor was there any sign of digestive leukocytosis. Casey et al. (19) also report that the heavier breeds of rabbits, as compared to the lighter breeds, have significantly higher total leukocyte counts, with the increase being primarily in basophils and monocytes. Casey (17) reports

that from his observation, it would appear that basopenia is associated with a state of low resistance and the exact relation of basopenia to the state of low resistance, whether of a casual or a concomitant nature is not known. Rosahn et al. (66) state that there are no significant differences in the leukocytes of males and females in regard to numbers. Bushnell and Bangs (16) report that the leukocyte count of 100 rabbits studied by them was 12,500 cells per cu. mm. Jackson and Stovall (42) observed a leukocyte count of 10,371 cells per cu. mm. for 22 rabbits. Hueper (40) reports an average of 8.100 cells per cu. mm. Reifenstein et al. (65) state the average leukocyte count of 14 New Zealand White and mixed rabbits was 9,449 cells per cu. mm. Wilson (85) reported the average leukocyte count to be 9,900 cells per cu. mm. Smith (75) reported an average of 11,797 leukocytes per cu. mm. with a range of 5,575 to 20,775 leukocytes per cu. mm. Nettlesmith (57) reported an average of 9,300 leukocytes per cu. mm. DeCourcey et al. (25) reported the average leukocyte count of ten male New Zealand White rabbits as being 9,886 leukocytes per cu. mm.

It is common knowledge that the heat regulating mechanism of an animal is subject to various external factors, such as exercise, food, excitement and rough handling and any or all of these factors will cause a rise of several tenths of a degree of temperature.

Winternitz and Pratt (86) state the average temperature for a normal rabbit ranges from 98.0° F. (36.6° C.) to 102.0° F. (38.8° C.). Seibert and Mendel (70) report the average of

40 rabbits for all seasons of the year, except the summer, to be 102.2° F. (39.05° C.). In addition, they found a normal daily variation of 1.0° F. (0.54° C.). Frothingham and Minot (33) found a range in the normal body temperature of 101.5° F. (38.6° C.) to 104.2° F. (40.1° C.), with an average of 103.1° F. (39.9° C.). They report no differences in the temperature between females and males. Richet (Frothingham and Minot, 33) reports an average of 103.2° F. (39.55° C.). Bock (Seibert and Mendel, 70) found an average temperature of 104.0° F. (40.0° C.). Krause (Moore, 53) reports that the experimental exercise in rabbits caused the temperature to rise from 102.2° F. (39.05° C.) to 104.1° F. (40.1° C.). Scott and Simon (69) report the temperature of 100 rabbits ranged from 100.8° F. (39.0° C.) to 103.6° F. (39.7° C.) with an average of 102.3° F. (38.1° C.). Pembrey (Frothingham and Minot, 33) gives the temperature range of 98.6° F. (37.0° C.) to 105.4° F. (40.8° C.) with an average of 101.7° F. (38.7° C.). Moore (52) found a range of 102.9° F. (39.4° C.) to 103.8° F. (39.9° C.) with an average of 103.4° F. (39.6° C.). In addition, it was found that the temperature of rabbits tied down fell 1.8° F. (1.0° C.) to 3.6° F. (2.0° C.). According to Moore (54), feeding the rabbit will cause a rise in temperature of 1.0° F. (0.54° C.). White (83) states that the normal rectal temperature for a rabbit is between 101.0° F. (37.2° C.) and 103.0° F. (39.4° C.).

MATERIALS AND METHODS

Phase I. The Body Temperature of Control Rabbits and Rabbits Affected with Conjunctivitis.

In many animal diseases the body temperature is elevated. This experiment was conducted to determine if there was a rise in the body temperature and the extent of the rise if it were present.

Ten rabbits were used in this experiment. They were of the Californian and New Zealand White breeds. Both males and females were used. Four of the rabbits were used as controls and the remaining six were either moderately or very seriously affected in one or both eyes with catarrhal or purulent conjunctivitis. The rabbits were fed a commercial feed which consisted of the following composition:

Crude protein (minimum)	15%
Crude fat "	2%
Crude fiber "	18%
Nitrogen Free Extract	46%

In addition these constituents were added:

Calcium carbonate	1.5%
Deflorinated phosphate	1.5%
Iodized salt	0.5%

In the investigation of the body temperature a standard rectal thermometer, graduated to 110° F. was used. The thermometer was inserted into the rectum beyond the grasp of the sphincter ani muscles to prevent the contraction of the sphincters forcing the mercurial column to a point higher than that resulting from the actual body temperature, Abbot (1).

The rabbits were handled in a manner to cause the least excitement. Excitement has been shown to cause an increase in the body temperature. The thermometer was cleaned and immersed in 70 per cent alcohol after taking the temperature of each rabbit to lessen the chance of infection. The thermometer was left in the rectum for two minutes. The temperature was taken each day for 21 days at the same time of day and recorded.

Phase II. The Transmissibility of Catarrhal and Purulent Conjunctivitis by Direct Contact.

Contagious diseases are frequently transmitted by direct contact from one animal to another. This experiment was conducted to determine if there was a possibility of conjunctivitis being transmitted from one rabbit to another by means of direct contact.

Eight rabbits were used in this experiment. They received the feed stated in Phase I. Adult rabbits of both sexes were used. They were of the Californian and New Zealand White breeds. Four normal rabbits showing no symptoms of conjunctivitis were used for the controls. The controls were examined for conjunctivitis by everting the lower and upper eyelids and observing the palpebral and bulbar conjunctivae and the conjunctival sac for lesions. Two rabbits showing catarrhal conjunctivitis and two rabbits showing purulent conjunctivitis were used as the source of infection. One normal rabbit was placed in a cage with one infected rabbit. The infected rabbits

and the normal rabbits were permitted to remain together in the same cages for a period of 90 days. Daily observations were made to observe for symptoms and lesions of conjunctivitis.

Phase III. The Transmissibility of Conjunctivitis by Direct Swab Method.

Secretions frequently contain the causative agent of a disease. When these secretions are cultivated on artificial media they may lose their pathogenicity. This experiment was conducted to reduce the chance of the organisms losing their virulence, and to determine if conjunctivitis could be transmitted in that manner.

Two infected rabbits were used for the source of the material. One of the rabbits was showing a catarrhal conjunctivitis and the other purulent conjunctivitis. Four rabbits that were healthy and free of conjunctivitis were used for the inoculation. Two of the normal rabbits were used for the inoculation for catarrhal conjunctivitis and the remaining two were used for the inoculation with the material from the purulent conjunctivitis case. Swabs were prepared by placing a cotton tip on wooden applicator sticks. The swabs were then immersed in 2 cc. of physiological saline solution and sterilized for 15 minutes at 15 lbs. pressure. The eyelids were everted by an assistant while the wet swabs were streaked across the eyelids, eyeball and conjunctival sac of the infected rabbits. These swabs were then streaked upon the eyeball and in the conjunctival sac and under the eyelids of the right eyes of the

normal rabbits. The left eyes served as controls. The inoculated rabbits were then observed each day for a period of 90 days for any symptoms of catarrhal or purulent conjunctivitis.

Phase IV. The Susceptibility of Guinea Pigs to the Causative Agent of Purulent Conjunctivitis From Rabbits with Conjunctivitis.

This phase was conducted to determine if other laboratory animals were susceptible to conjunctivitis found in rabbits.

Four healthy guinea pigs were selected for this experiment. The material for the inoculations was taken from the eyes of a rabbit that showed bilateral purulent conjunctivitis. A cotton swab was immersed in physiological saline and then streaked across the eyes and under each of the eyelids and in the conjunctival sacs. After gathering the exudate on the swab, it was then placed in a tube containing 8 cc. of physiological saline that was sterile. Using a sterile 5 cc. glass syringe and a 20-gauge, two inch needle, 2 cc. of the suspension were injected subcutaneously into each of two guinea pigs, in an aseptic manner. Two cubic centimeters of the same material were injected intraperitoneally, in an aseptic manner, into each of two guinea pigs. The guinea pigs were observed daily for a period of three weeks for any changes.

Phase V. The Hemogram in Conjunctivitis of The Rabbit.

In reviewing the literature, no reference was found that any work had been done on the hematology of the rabbit affect-

ed with conjunctivitis. This experiment was undertaken to determine if conjunctivitis was viral or bacterial in nature. It is known that conditions such as swine erysipelas cause a leukocytosis, and others such as hog cholera cause a leukopenia.

The rabbits used in this experiment were the control animals previously described and infected animals that had been submitted to the diagnostic laboratory.

The rabbits were bled from the heart with an 18-gauge, two inch needle attached to a 10 cc. syringe. Five cubic centimeters of blood were withdrawn and mixed with oxalate as an anticoagulant. The anticoagulant used was a dry mixture of 1.2 grams of ammonium oxalate and 0.8 gram of potassium oxalate in 100 ml. of distilled water (Heller and Paul, 39). The oxalate was added to a 10 cc. glass tube in the amount of 0.7 cc. This was then dried in an oven.

Standard methods were used for the determination of the total erythrocytes and leukocytes. In the total erythrocyte determination the oxalated blood was drawn up to the 0.5 mark of the red cell diluting pipette and Hayem's diluting fluid was drawn to the 101 mark of the pipette, thus making a dilution of 1:200. Hayem's solution consists of the following:

Sodium chloride	1.0 gram
Sodium sulfate	5.0 grams
Mercuric chloride	0.5 gram
Distilled water	200 ml.

The pipette was rotated while being filled. The blood and the diluting fluid in the pipette were shaken for one minute on an electrical pipette shaker. The cover glass was then placed in

position on the counting chamber on the hemacytometer. Three or four drops of fluid were discarded from the pipette, and the tip of the pipette was touched to the edge of the platform of the hemacytometer; care being taken not to let any of the fluid escape or overflow into the surrounding moat, and that there was no air under the cover glass. The fluid was allowed to stand for a few minutes before examination.

The hemacytometer was then placed under the microscope and the 16 mm. objective was used to locate the ruled lines and to determine if the cells were evenly distributed over the chamber. The 4 mm. objective was used for the actual count. Both chambers of the hemacytometer were filled from separate pipettes containing blood from the same animal.

In counting the erythrocytes, 80 of the smaller squares of the hemacytometer were counted. This was accomplished by counting the erythrocytes in the four corner squares and one center square of the central area, which is finely ruled, in the hemacytometer. There are 16 small squares in one big square and this is bordered on all sides by a double line. On the top row, the cells were counted from left to right, on the second row from right to left etc., until the cells in the four rows were counted. The cells lying on the left and upper outside double lines were included in the count, while those on the right and lower outside double lines were excluded from the count.

The counts were made on both sides of the hemacytometer and averaged. If the counts varied too much, new counts were

made by taking another sample from each of the pipettes. The square root of the average of the two counts was extracted in determing whether excessive variation of the counts occured. If this number was greater than the difference between the two counts and the average, the cells were considered to be from the same population.

In the calculation of the total erythrocyte count, the total number of cells counted in 80 small squares divided by 80, determined the average number of erythrocytes per small square or in 0.00025 cu. mm. The number of cells counted in 0.00025 cu. mm. was multiplied by 4,000 to determine the number of cells present in 1 cu. mm. of undiluted blood. For routine work and only when the dilution was 1:200, four ciphers were added to the number of cells in 80 small squares.

Leukocytes were enumerated in a similar manner as erythrocytes. The blood was drawn to the 0.5 mark of the leukocyte diluting pipette. The diluting fluid was then drawn to the 11 mark of the pipette, thus making a dilution of 1:20. The diluting fluid consisted of the following:

Concentrated HCl	1.2 ml.
Distilled water	98.8 ml.

The diluting fluid was filtered before using. This diluting fluid produces hemolysis of the erythrocytes. The blood and the diluting fluid were shaken for one minute by an electrical pipette shaker before making the count. Three or four drops were discarded and care was taken not to overfill the counting chambers. The two chambers of the Neubauer hemacytometer were

filled with separate pipettes containing blood from the same animal. The cells were examined under the 16 mm. objective to determine if there was an even distribution on the counting chamber. The leukocytes were counted in the four large corner squares of the hemacytometer. Each of these four square millimeter areas was subdivided into 16 squares to facilitate counting. The total number of leukocytes in the four corner squares was multiplied by 50 to determine the number in one cubic millimeter of undiluted blood.

Blood was collected and mixed with an anticoagulant (oxalate) for the sedimentation rate. The blood was then placed in the Wintrobe hematocrit tube to the 10 cm. mark with a capillary pipette. The tube was held vertically in a rack. The distance that the erythrocytes fell in 15, 30 and 60 minutes was noted. The sedimentation test was carried out at room temperature. Duplicates were made on each blood sample.

The volumes of packed red cells were determined after the sedimentation rates were made. The hematocrit tubes were centrifugalized at 3,000 r.p.m. for 30 minutes. The object of the centrifugation was to secure complete packing of the corpuscles in the hematocrit tubes. The volume of packed red cells was read directly from the scale on the hematocrit tube. This number was multiplied by ten, to give the volume in per cent.

The hemoglobin determination was made by the use of the Fisher Electro-hemometer. Twenty cubic milimeters of blood in 5 ml. of 0.1 N HCl were used to form the acid hematin.

This mixture was allowed to stand for five minutes. The hemometer was adjusted to zero with the blank tube of distilled water. The acid hematin tube was then place in the hemometer and the reading was taken direct from the scale on the hemometer. The results were expressed in grams of hemoglobin per 100 ml. of blood.

The specific gravity of the whole blood was determined by the method described by Phillips et al. (63). The procedure consists of letting drops of whole blood fall into a graded series of copper sulfate solutions in which the specific gravity is known, and noting whether the drop of blood rises or falls in the solution. The drop of blood entering the solution becomes encased in a sack of copper-proteinate and remains as such without change of gravity for 15 to 20 seconds. During this time its rise to the surface or fall to the bottom reveals its specific gravity relative to that of the solution. The specific gravity of the copper sulfate in which the drop neither falls nor rises to the surface corresponds to the specific gravity of the blood sample used.

The differential leukocyte counts were made by staining the blood smear with Wright's stain for three minutes. The buffer solution was allowed to remain for five minutes. The "four-field meander" method described by Gradwohl (36) was used in making the counts of 100 cells.

The mean corpuscular volume was determined by the following formula:

M.C.V.= $\frac{\text{Volume of packed erythrocytes per 1000 cc. of blood}}{\text{Red cell count, millions per cu. mm. of blood}}$

Phase VI. The Anatomical Location of the Nasolacrimal Duct and Canal.

The literature on the anatomy of the nasolacrimal canal and duct in the rabbit is very limited. Three normal rabbits were used in this phase.

Diluted aqueous fuchsin was placed in the conjunctival sac to assist in the determination of the course of the nasolacrimal canal and duct. When the aqueous fuchsin was observed in the nares, euthanazia was performed and the heads were removed from the rabbits. The skin and muscle was removed from the head and the mandible was disarticulated. Specimen Number I was macerated by placing it in water and allowing it to stand until all of the remaining flesh would cleave from the skull. Specimen Number II was placed in concentrated formalin and allowed to remain for 18 hours to fix the tissue, after this time it was placed in the deep freezer and frozen for 24 hours to facilitate sectioning. Specimen Number III was allowed to remain in the natural state.

The location of the punctum lacrimalis was determined from Specimen Number I. Specimen Number II was divided in half by sawing longitudinally from a point one half way between the occipital condyles and the cleft of the nares. One half of the specimen was then sawed transversely into sections approximately one-half inch apart. A wire probe was

placed through the nasolacrimal duct, thus connecting the sections. Specimen Number III was dissected in situ.

Phase VII. The Determination of the Occlusion of the Nasolacrimal Duct.

Epiphora is usually observed in rabbits affected with purulent conjunctivitis. This phase was conducted to determine if the nasolacrimal duct was occluded in the infected rabbits.

Ten rabbits were used for the determination. Five of the rabbits were normal and healthy and they were used for the controls. The remaining five were showing the characteristic epiphora of purulent conjunctivitis. The eyelids were everted while a few drops of aqueous fuchsin were instilled into the conjunctival sac with an eye dropper. The eyelids were then permitted to return to their natural position and the rabbits were observed very closely for the appearance of the dye in the nares.

Phase VIII. The Histopathology of the Conjunctiva.

The literature reviewed did not mention the histopathology of the conjunctivae in rabbits affected with purulent conjunctivitis. This phase was conducted to determine if there were any microscopic changes in the conjunctiva of the rabbit with purulent conjunctivitis.

One normal rabbit and one rabbit showing purulent conjunctivitis were used for this phase. Euthanasia was performed on the rabbits. The eyeball and adnexa were enucleated

by suturing the eyelids together and making an elliptical incision from the medial canthus to the lateral canthus of the eye. The eyeball and adnexa were removed by applying traction upon the sutures while dissecting around the eyeball inside of the bony orbit.

The eyeball and adnexa were then placed in buffered formalin in preparation for sectioning. The conjunctiva was removed from the eyeball by the use of a tissue knife. Dehydration of the conjunctiva was accomplished by the use of alcohol in ascending strengths from 70 per cent to absolute alcohol. The tissues were cleared using cedar-wood oil. Infiltration of the tissues was accomplished by the use of paraffin and cedar-wood oil. The tissues were embedded in paraffin and then mounted on wooden blocks. The blocks were cooled and then sectioned eight microns thick. The tissue section was then fixed to a glass slide by the use of albumen solution. The sections were "brought to water" and stained with hematoxylin and eosin. The sections were then dehydrated, cleared and mounted. The sections of the normal and infected conjunctiva were then examined for pathological changes.

Phase IX. The Bacteriological Examination of the Eye.

This phase was conducted to determine the normal bacterial flora of the eye of conjunctivitis-free rabbits and rabbits showing catarrhal or purulent conjunctivitis.

Ten normal rabbits and ten rabbits with either catarrhal

or purulent conjunctivitis were used for this phase. The normal rabbits were determined free of conjunctivitis by examination of the palpebral and bulbar conjunctiva in the manner previously described. The infected rabbits were those submitted to the diagnostic laboratory for examination.

Sterile cotton swabs were prepared as mentioned before. The swabs were streaked across the eyeball, under the eyelids and in the conjunctival sac and were then streaked on the different media listed below. The contamination from the skin was held to a minimum by everting the eyelids and being careful not to touch the palpebral fur with the swabs.

Tryptose Dextrose Blood Agar was used for the initial isolation of the organisms except where Sabouraud's media was used. The Tryptose Dextrose Blood Agar was prepared as follows:

Bacto Tryptose	20 grams
Bacto Dextrose	1 gram
Sodium Chloride	5 grams
Bacto Agar	20 grams

To rehydrate the medium, the above constituents were suspended in one liter of cold distilled water. The mixture was then heated to boiling to dissolve the medium completely. It was adjusted to pH 7.2. Following this, the medium was bottled and sterilized in the autoclave for 15 minutes at 15 pounds pressure (121.6° C.). It was then cooled to 42-45° C, and five per cent sterile defibrinated blood was added to the medium. Rabbit and bovine blood were used for enrichment. The mixture was poured into sterile Petri plates and allowed

to incubate at 37° C. for 12 to 18 hours to insure sterility.

Sabouraud Dextrose Agar was incorporated into the study to determine if molds or fungi were present in the eye, especially in the eyes of rabbits affected with catarrhal and purulent conjunctivitis. Two swabs from the normal rabbit's eyes were streaked on this medium. Sabouraud Dextrose Agar was prepared as follows:

Difco-Necpeptone	10 grams
Bacto-Dextrose	40 grams
Bacto-Agar	15 grams

To rehydrate the medium, 65 grams of Bacto-Sabouraud Dextrose Agar were suspended in one liter of cold distilled water and heated to boiling to dissolve the medium completely. The medium was adjusted to pH 5.6. The medium was then distributed into flasks and sterilized for 15 minutes at 15 pounds pressure (121.0° C.), and then cooled to 45-50° C. and poured into sterile Petri plates. A plate was allowed to incubate at 37° C. with the cultures to insure sterility.

The blood agar plates were incubated at 37° C. for 24 to 96 hours following streaking with the cotton swabs. The colonies present were studied as to the general morphology and subcultured on blood agar plates to obtain a pure culture. The subcultures were incubated at 37.0° C. for 24 to 96 hours. A Gram stain was made of isolated colonies and if a pure culture was obtained, they were inoculated into tryptose broth. Subcultures were made if the colonies were not pure. The tryptose broth was incubated at 37.0° C. for 24 to 96 hours and the

growth was observed. Gram stains were made of these to determine if there was a pure culture. Bergey (14) was consulted and the cultures were identified by the bacteriological methods listed under the different families.

RESULTS AND DISCUSSION

Symptoms of Conjunctivitis

Conjunctivitis usually appears when the rabbit is eight months to one year of age or older. It may affect either sex, but from clinical observations, it appears to affect more bucks than does. Conjunctivitis is most prevalent during the spring months when the weather is damp and windy. The condition is the least prevalent during the summer months. All breeds of rabbits may be affected, but the New Zealand White and the Californian breed seem to be the most susceptible. Data collected from various breeders indicate that the malady has been a problem to the rabbit breeder for approximately fifteen years. Some breeders are of the opinion that some factor of heridity may be associated with the condition. They have observed that three generations of rabbits have shown the typical symptoms, and in each succeeding generation, the symptoms are more pronounced and severe.

One of the first symptoms noticed in conjunctivitis in the rabbit is a slight reddening and inflammation of the palpebral conjunctiva. The conjunctiva may show some signs of chemosis. The bulbar conjunctiva may not show as much reddening and

chemosis as the palpebral conjunctiva. Active hyperemia of the scleral vessels usually accompanies the condition. A thin, clear catarrhal exudate appears in the medial canthus of the eye 24 to 48 hours after the initial symptoms. One or both eyes may be affected and the rabbit may rub the eyes with the front feet. The discharge from the eye may be slight or profuse. The fur of the lower eyelid becomes moist or wet. Photophobia is not noticeable. The appetite remains unchanged throughout the condition.

After the discharge from the eyes has progressed for one to two weeks, the fur along the side of the face below the eye begins to fall out, leaving a denuded area. (Plates I and II). This area may extend from the medial canthus of the eye to the nostril, depending upon the amount of infection. Flakes of dried purulent exudate are later seen alon the side of the face. A small amount of purulent exudate may remain in the conjunctival sac and the medial canthus of the eye. The condition apparently begins its course as a catarrhal conjunctivitis and later when the inflammation is more intense or severe, a purulent form develops, hence the name watery eyes, weeping eyes or running (pus) eyes as laymen speak of the condition. The condition may terminate without treatment, or the rabbit may succumb. The gross post mortem finding are not characteristic, except for the eye and the face. The nasolacrimal duct may be occluded. There is no great elevation in the body temperature. If the rabbit recovers, the hair grows back and there is no

EXPLANATION OF PLATE I

Close-up or rabbit with denuded area from the secretions of conjunctivitis.

PLATE I

EXPLANATION OF PLATE II

Rabbit showing characteristic epiphora of conjunctivitis.

PLATE II

apparent damage done to the tissues of the face or to the eyes. The course of the disease varies with the congestion present and the amount of discharge from the eyes. An epiphora is seen in most cases, and when this subsides, the rabbit is apparently recovering from the condition.

Phase I. The Body Temperature of Control Rabbits and Rabbits Affected with Conjunctivitis.

Plate III shows the mean temperature curves of the control rabbits and the rabbits affected with conjunctivitis. The average for the control rabbits was 103.48° F. (39.67° C.) with a range of 103.0° F. (39.40° C.) to 104.07° F. (40.07° C.). This average compares favorably to the temperature mean reported by Moore (53) as he reports an average of 103.4° F. (39.6° C.).

The average for the infected rabbits was 103.78° F. (39.83° C.) with a range of 103.1° F. (39.46° C.) to 104.6° F. (40.29° C.). The difference between the two average was 0.30° F. (0.16° C.).

There were no temperature differences noted between the male and female rabbits. The temperature was slightly elevated during the first few days of the preliminary temperaturing. This was probably due to the excitement of the rabbits and their being accustomed to being handled, as once they were accustomed to the procedure, there was not any excitement, and consequently a true reading could be taken.

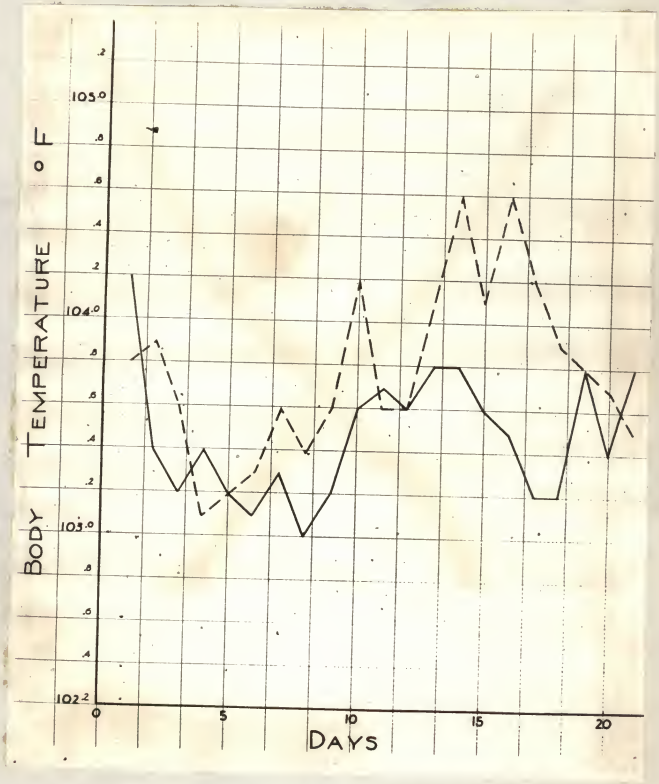
The infected rabbits did not show any marked rise in

EXPLANATION OF PLATE III

Means of the daily body temperatures of six rabbits with conjunctivitis and four normal rabbits.

The broken line indicates the infected rabbits and the continuous line indicates the normal rabbits.

PLATE III



temperature as compared to the control rabbits. The condition is not considered febrile in the light of the difference of the mean temperatures of the control and infected animals, as the rise in temperature was only 0.30° F. (0.16° C.).

Environmental factors often have an influence on the body temperature. For example, during a hot day the temperature of a hog will rise several degrees. This does not seem to hold true of the rabbit however, as the temperatures were not observed to rise markedly during the very hot days of August. The temperatures were not lowered over 0.5° F. when air conditioning was commenced in the room in which the rabbits were housed.

Boyd (13) states the the temperature of the human body remains constant even if there is a change in the environmental temperature. The regulatory mechanism breaks down under the increased strain and the body temperature rises, if the body is exposed to excessive heat. Dukes (30) states that heat regulation is developed only in those animals with a highly organized nervous system.

Phase II. The Transmissibility of Catarrhal and Purulent Conjunctivitis by Direct Contact.

The normal rabbits failed to show any symptoms of either catarrhal or purulent conjunctivitis at the termination of the 90 day contact period. This would indicate that neither catarrhal nor purulent conjunctivitis was transmissible from one rabbit to another by direct contact.

The catarrhal form of conjunctivitis is the first to develop in the eye, this is usually followed by the purulent form. This phase was carried out to determine if either form was transmissible, but more especially to determine if the rabbit with the catarrhal form was capable of transmitting the etiological agent to a normal rabbit. The possibility of the causative agent leaving the eye or becoming inactive was considered in the purulent form. Therefore, the catarrhal form may have contained the causative agent. This is supported by Axenfeld (6) and he states that in many forms of conjunctivitis, the characteristic findings can be obtained only during the height of a disease. He states in addition, that the causal agent can disappear rapidly although the discharge may still be profuse. Trumbower and Creech (79) state that infectious catarrhal conjunctivitis appears in an enzootic form and may affect all newly purchased animals.

A number of factors influence lysozyme, chiefly the hydrogen ion concentration, the pH being between 6.0 and 7.4 depending on the bacterial flora, according to Adler (2). The lysozyme present could be in sufficient concentration to affect the bacteria present before the purulent state was reached.

Sherwood (73) lists a conjunctival test for allergy due to the pollen of hay fever which consists of dropping a dilute solution of the pollen into the conjunctival sac of the human eye. A positive reaction is indicated by a diffuse reddening of the conjunctiva accompanied by pruritis and a watery discharge.

The 90 day contact period was considered ample time for any possibility of transmitting conjunctivitis from one rabbit to another.

Phase III. The Transmissibility of Conjunctivitis by Direct Swab Method.

The day following the swabbing of the eyes the four rabbits subjected to the test showed a slight active hyperemia of the conjunctivae. This was probably due to the minor irritation of the swab as it was streaked across the conjunctiva several times in attempting to release the causative factor. The hyperemia disappeared on the third day. The rabbits were observed very closely for any symptoms of conjunctivitis during the 90 day observation period. After the initial hyperemia subsided, there were no symptoms observed in the rabbits that were subjected to the material from catarrhal conjunctivitis and purulent conjunctivitis. It is well known that secretions frequently contain the causative agent of a disease, therefore, it seems possible that if the causative agent was found in the secretions from the conjunctivitis, it would be transmitted this way. In addition, the organism would not lose its virulence if it were transmitted directly. If a symbiosis existed between organisms causing conjunctivitis in the eye, this procedure should, in all probability, demonstrate this condition as several organisms have been found from a single swabbing of the eye. Fistulous withers and poll evil in the equine are good examples of symbiosis of bacteria. This phase

would indicate that the secretions probably do not contain any infectious organism that is transmissible from one rabbit to another and causing conjunctivitis.

Phase IV. The Susceptibility of Guinea Pigs to the Causative Agent of Purulent Conjunctivitis from Rabbits with Conjunctivitis.

There were no noticeable symptoms in either the guinea pigs injected subcutaneously or intraperitoneally during the 21 day examination period. This period was chosen because of the possibility of the causative agent being slow to develop within the body as is the case with brucellosis in the bovine, and to produce symptoms of the conjunctivitis or a septicemia from the purulent material. Conjunctivitis has never been observed in guinea pigs in the diagnostic laboratory colony. The guinea pigs are fed the same ration as the rabbits; therefore, if conjunctivitis was of dietary origin in the rabbit, it would be possible for the same symptoms to develop in the guinea pig colony. This phase would indicate that the guinea pigs examined were not susceptible to rabbit conjunctivitis. It would also indicate that the exudate from rabbits showing purulent conjunctivitis did not have any effect on the health of the guinea pigs. There was no local inflammation at the site of the injection, conjunctivitis or systemic involvement.

Phase V. The Hemogram in Conjunctivitis of the Rabbit.

The mean difference in mean corpuscular volume between

the control and infected animals approached the five per cent level of significance. Wintrobe et al. (88) list the average mean corpuscular volume for the rabbit as 64 plus or minus four. The mean of the control rabbits in Table I was 59.41. From the increase of the mean corpuscular volume in the infected rabbits it may be assumed that a macrocytosis exists to some degree. Sturgis (77) stated that in pernicious anemia, the mean corpuscular volume is increased and, in general, the more severe the anemia the greater the mean corpuscular volume. Kolmer et al. (47) state that if the cells are larger than normal, the condition may be called "macrocytosis". There is no apparent anemia in either the control or the infected rabbits as shown by Table I. The mean erythrocyte count of the control animals was 6,550,000 per cu. mm. This is in the range reported by Wintrobe et al. (88) and Bushnell and Bangs (16). The infected rabbits had a mean of 5,265,000 per cu. mm. which is in the normal range as reported by Rosahn et al. (66).

The mean difference between the total erythrocyte counts approached the five per cent level of significance. However, in reviewing the hemoglobin values (Table 3.) it was found that these values were not decreased in the infected animals; therefore, it is likely that there is little significance attached to the decrease in the total erythrocytes in the infected animals. On the other hand, it is possible to have a normal hemoglobin content with a decrease in the number of erythrocytes (87, 32).

In table 2. the mean difference in the sedimentation rates

Table 1. Ranges and means of mean corpuscular volume and total red blood corpuscles of conjunctivitis-free rabbits and rabbits with conjunctivitis.

Group	Number Rabbits	M. C. V. cu. mic. Range	Mean	Total r. b. c. 10^6 Range	Mean
Control	5	40.78-78.05	59.41	4.93-8.41	6.530
Infected	6	60.22-101.03	80.62	3.08-6.43	5.265

Table 2. Ranges and means of the sedimentation rate and hematocrit of conjunctivitis-free rabbits and rabbits with conjunctivitis.

Group	Number Rabbits	Sed. Rate mm./hr. Range	Mean	Hematocrit per cent Range	Mean
Control	5	0.3-1.0	0.6	34.3-42.0	37.6
Infected	6	1.0-1.6	1.2	33.0-41.0	38.3

Table 3. Ranges and means of the hemoglobin and specific gravity values of conjunctivitis-free rabbits and rabbits with conjunctivitis.

Group	Number Rabbits	Hemoglobin gms./100 ml. Range	Mean	Specific Gravity Range	Mean
Control	3	13.0-13.8	13.3	1.054-1.056	1.055
Infected	3	13.0-14.5	13.7	1.056-1.058	1.056

between the infected and control rabbits was highly significant. The sedimentation rate gives information of a general character. The rate under normal conditions is fairly constant, but in some bacterial and viral diseases there is a marked increase. The true nature of the sedimentation rate is not fully understood. Probably the most important factor is the ability of the erythrocytes to form aggregates. The larger the aggregates, the greater the rate of fall. Changes of the protein in the plasma may affect the aggregates according to Kolmer et al. (47). Todd and Sanford (78) stated that the phenomenon is apparently associated with the ratio of albumin, globulin and fibrinogen in the plasma. They also state that the rate is increased in acute localized inflammations. This would follow the results of this experiment as the rabbits do have an acute localized inflammation of the eye in conjunctivitis.

The hematocrit values show no significant mean difference between the normal and the infected rabbits. The hematocrit is a procedure of great value in diagnosis and should be used in routine investigations, according to Wintrobe (87). The hematocrit is very valuable in determining the degree of anemia present in an animal.

The hemoglobin values did not show any significant difference among means (Table 3.). The results were higher than the averages reported by Gardner (34). She gives the average of the reported values as 12.07 grams per 100 ml. The amount of hemoglobin in the control rabbits averaged 13.3 gm. per

100 ml., in comparison to the infected which was 13.7 gm. per 100 ml. It is reasonable to assume that the method used in the hemoglobin determination would have some effect on the results reported. No difference was noted in the hemoglobin values for the different sexes of rabbits.

The means of the specific gravity of the blood of both the control and infected rabbits were 1.055 and 1.056 respectively. Conjunctivitis did not alter the specific gravity of the blood. Dougherty and White (28) reported an average of 1.053 plus or minus 0.0009. The specific gravity of blood depends upon such factors as quantity and hemoglobin content of the erythrocytes and the protein content of the plasma (87). The copper sulfate method used has many advantages over the other types of apparatus to measure the specific gravity.

There was no mean significant difference in the total leukocyte counts between the infected rabbits and the control rabbits (Table 4.). This would help support the view that conjunctivitis is neither bacterial nor viral in nature. Bacterial diseases such as swine erysipelas will cause a leukocytosis while, on the other hand, viral diseases such as hog cholera will cause a leukopenia. The finding of the total leukocyte counts are consistent with the ranges reported by other investigators (40,75,57).

Several factors may influence the number of leukocytes in the circulating blood. For example, various drugs and pregnancy may cause leukocytosis. Factors such as prolonged undulant

Table 4. Ranges and means of differential leukocyte counts of conjunctivitis-free rabbits and rabbits with conjunctivitis.

	Control		Infected	
Number rabbits	5		6	
Tot. leukocyte	7,711[a] (6,385-8,440)[c]		7,658 (5,350-11,150)	
Mature pseudo eos. and eosinophil	41.4[b] (26-47)	3,192[a] (2,775-3,624)	41.0 (27-78)	3,139 (2,067-5,973)
Small lymphocyte	33.8 (28-40)	2,606 (2,159-3,084)	42.0 (10-62)	3,216 (765-4,747)
Large lymphocyte and monocyte	14.4 (11-19)	1,110 (848-1,465)	11.0 (8-17)	842 (612-1,301)
Immature pseudo eos. (Stab and Juv.)	8.0 (3-10)	616 (221-771)	1.08 (0-4)	82 (0-306)
Basophil	3.0 (0-5)	231 (0-385)	1.5 (0-4)	114 (0-306)

[a] mean number [b] mean per cent [c] range

fever (87), influenza, malaria, anaphylactic shock and in the early stages of reaction to foreign protein may cause a leukopenia.

Phase VI. The Anatomical Location of the Nasolacrimal Duct and Canal.

The location of the punctum lacrimalis was determined from the specimens examined. The orbital opening of the nasolacrimal canal was found on the ventral border of the membrana nictitans, at its junction with the lower eyelid. It is readily discernable when the membrana nictitans is retracted outward and

downward with forceps. The osseous part is found at the base of the hamulus lacrimalis. The canal is osseous for approximately one inch in its course from the corneal surface of the eye to the anterior part of the nasal fossa. It passes downward medial to the lacrimal and maxilla bones, and opens into the anterior portion of the nasal fossa. The opening into the nares was difficult to ascertain due to the cleft nose and small nares. Sisson and Grossman (74) state that in the horse, it is not rare to find one or two accessory orifices farther back in the nasal cavity. This was not observed in the rabbits examined.

Phase VII. The Determination of the Occlusion of the Nasolacrimal Duct.

Epiphora is the result of the nasolacrimal duct becoming occluded with the purulent exudate that is formed in the later stages of a rabbit showing purulent conjunctivitis. In the catarrhal form of conjunctivitis, the exudate is not purulent and does not occlude the nasolacrimal duct. The five rabbits that were used which were free of conjunctivitis did not show any occlusion of the nasolacrimal duct. This was ascertained by placing aqueous fuchsin in the conjunctival sac and observing it appear at the nares in approximately 30 seconds. The aqueous fuchsin was placed in the conjunctival sacs of the five rabbits showing purulent conjunctivitis, and it appeared in the nares on the side of the non-affected eye if the conjunctivitis was unilateral, or if both eyes were affected, it did not appear.

This was demonstrable proof that the nasolacrimal duct was occluded in purulent conjunctivitis. The epiphora continues as long as the nasolacrimal duct is occluded.

The nasolacrimal duct may be occluded by mechanical blockage, by foreign bodies such as grass seeds and it may be congenital. Belschner (9) states that ophthalmia in sheep, caused by the grass seeds getting into the eyes, is common.

Phase VIII. The Histopathology of the Conjunctiva.

The sections of the normal conjunctiva were compared with the sections of the conjunctiva of a rabbit affected with purulent conjunctivitis. Figures 1 and 2 show these two slides. Comparing the abnormal to the normal, it is seen that epithelial plaques are present in the sub-epithelium. There are signs of edema or chemosis present as evidenced by the space between the cells. There is a slight lymphocytic infiltration present. The stratified squamous epithelium is still intact and does not appear to be damaged.

According to Runnells (67) the metaplasia from cuboidal and columnar epithelium to keratinized epithelium is often accompanied by inflammation. This is often seen in vitamin A deficiency. A-avitaminosis is seen in calves and the eye lesions are frequent in immature animals, with catarrhal conjunctivitis and even blindness being present at times. Wolbach (89) considered the keratinization as an attempt to repair the epithelium following atrophy. According to Seifried, as cited by Hutyra et al. (41), the hyperkeratinization of the pavement

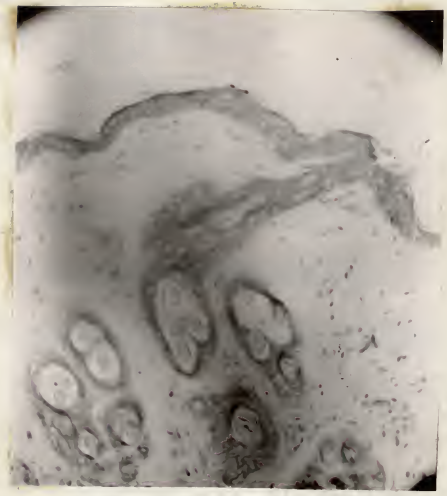


Fig. 1. Section of normal conjunctiva.

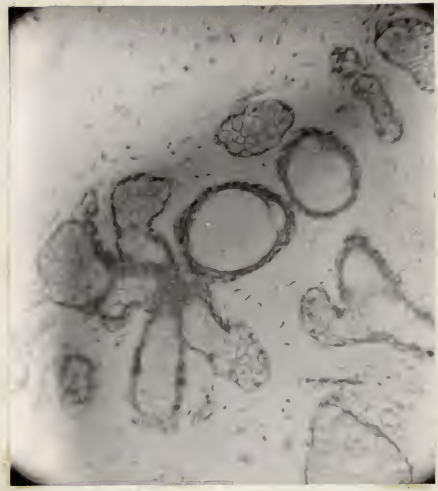


Fig. 2. Section of the conjunctiva of rabbit affected with purulent conjunctivitis.

epithelium resolves under the influence of vitamin A, and the morphological structure returns to normal.

Willis (84) states that vitamin A deficiency may cause squamous metaplasia and increased liability to bacterial infection. Anderson (4) states that the epithelial cells involved undergo atrophy, reparative proliferation of the basal layer of the epithelium, and replacement by stratified keratinized epithelium.

Phase IX. The Bacteriological Examination of the Eye.

Determinative studies revealed the following bacteria to be present in the eye of rabbits showing no conjunctivitis, Sarcina lutea, Bacillus subtilis, Staphylococcus albus, and Micrococcus epidermidis. In infected rabbits the above mentioned bacteria have, on occasion, been isolated and, in addition, Corynebacterium xerose has been isolated.

Hagen (38) states that in the limited work done on watery eye, the indication of a mixed flora is involved--namely, a diphtheroid, a micrococcus and a Pasteurella having been isolated from this condition.

The conjunctival sac has an external opening, therefore it is to be expected that the organisms present are also found in the air, the fur surrounding the eye and anything with which the eye may come in contact. The bacterial flora of the eye is in a dynamic state--with the possibility of changing within a very short period of time. It has been found that the conjunctiva of newborn infants is sterile and after ten days many bacteria

could be demonstrated according to Axenfeld (6).

The bactericidal effect of lysozyme, a colloidal enzyme, found in the tears is worthy of note as this enzyme undoubtedly has a deleterious effect on many of the bacteria present in the eye. The movement of the eyelids has an important function in the cleansing action upon the eye. It is known that the bandaging of an eye will oftentimes increase the numbers of the organisms present as the eyelids are not permitted to move and thus have a cleansing effect upon the eye. The eyelids also assist in moving the bacteria toward the nasolacrimal duct.

SUMMARY AND CONCLUSIONS

Phase I. The Body Temperature of Control Rabbits and Rabbits Affected with Conjunctivitis.

1. The average temperature for the control rabbits was 103.48° F. (39.67° C.).

2. The average temperature for the infected rabbits was 103.78° F. (39.83° C.).

3. The difference between the averages of the control and infected animals was 0.30° F. (0.16° C.).

4. There were no differences noted between male and female rabbits.

5. Conjunctivitis in the rabbit is not considered a febrile disease.

Phase II. The Transmissibility of Catarrhal and Purulent Conjunctivitis by Direct Contact.

1. The control animals failed to develop conjunctivitis within the 90 day contact period.

2. The indications are that neither catarrhal nor purulent conjunctivitis are transmissible from rabbit to rabbit by direct contact.

Phase III. The Transmissibility of Conjunctivitis by Direct Swab Method.

1. An initial active hyperemia of the conjunctiva was noticed following swabbing of the eyes. This subsided in 72 hours following the swabbing of the eyes.

2. After the initial active hyperemia subsided, there were no other symptoms of conjunctivitis observed during the 90 day observation period.

3. This phase would indicate that the secretions did not contain the causative agent of conjunctivitis.

Phase IV. The Susceptibility of Guinea Pigs to the Causative Agent of Purulent Conjunctivitis from Rabbits with Conjunctivitis.

1. There were no noticeable symptoms in the guinea pigs injected either subcutaneously or intraperitoneally with the exudate from the eyes of rabbits with conjunctivitis.

2. This phase would indicate that the guinea pigs examined were not susceptible to rabbit conjunctivitis.

3. The exudate from the conjunctiva of rabbits had no systemic effect on the guinea pigs.

Phase V. The Hemogram in Conjunctivitis of the Rabbit.

1. The five per cent level of significance was approached with the mean difference of the mean corpuscular volume between normal and infected animals.

2. A macrocytosis existed to some degree as evidenced by the increase of the mean corpuscular volume of the blood.

3. The erythrocyte counts of the normal rabbits fell in the normal ranges listed by different investigators.

4. The mean difference between the total erythrocyte counts of the control and infected rabbits approached the five per cent level of significance. However, the hemoglobin levels were not decreased in the infected animals, so the decrease in the total erythrocytes of the infected animals did not have too much importance.

5. No apparent anemia existed in either the control animals or the infected animals.

6. The mean difference in sedimentation rate between the infected and control animals was highly significant.

7. The hematocrit values showed no significant mean difference among or between the two groups of rabbits.

8. The hemoglobin values did not show any significant difference among the means of control and infected animals.

9. Conjunctivitis did not alter the specific gravity of the blood of the rabbits.

10. The total leukocyte counts of the control and infected animals failed to show any mean significant difference.

Phase VI. The Anatomical Location of the Nasolacrimal Duct and Canal.

1. The orbital opening of the nasolacrimal canal is on the ventral border of the palpebra tertia at its junction with the lower eyelid.

2. The osseous opening is at the base of the hamulus lacrimalis.

3. The nasolacrimal duct passes from the corneal surface of the eye to the anterior part of the nares.

Phase VII. The Determination of the Occlusion of the Nasolacrimal Duct.

1. Epiphora is the result of the nasolacrimal duct becoming occluded.

2. The normal rabbits did not show any occlusion as compared with the abnormal rabbits which demonstrated occlusion.

Phase VIII. The Histopathology of the Conjunctiva.

1. Epithelial plaques were present in the sub-epithelial tissue of the conjunctiva of the infected rabbits.

2. Edema and chemosis were present and there was a slight lymphocytic infiltration of the abnormal conjunctiva.

3. The epithelium appeared to be unchanged.

Phase IX. The Bacteriological Examination of the Eye.

1. The following bacteria were found to be present in the eyes of rabbits showing no conjunctivitis:

a. Sarcina lutea
b. Bacillus subtilis
c. Staphylococcus albus
d. Micrococcus epidermidis

2. In infected rabbits, the above mentioned bacteria have been found. In addition, Corynebacterium xerose has been isolated.

3. The bacterial flora of the eye is in a dynamic state.

4. It is believed that a possibility of several bacteria being the etiological factor of conjunctivitis in the rabbit.

5. Additional work needs to be conducted in the study of the bacterial flora of the rabbit's eye.

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CATARRHAL AND PURULENT CONJUNCTIVITIS IN THE NEW ZEALAND WHITE AND CALIFORNIAN RABBITS

by

GEORGE EDWIN KING

B. S., Kansas State College of Agriculture and Applied Science, 1953
D. V. M., Kansas State College of Agriculture and Applied Science, 1955

AN ABSTRACT

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Conjunctivitis is a serious and economical problem in rabbitries. The condition is also referred to as watery eyes, weeping eyes or running (pus) eyes. The catarrhal or purulent form may be present in the rabbit's eye. The condition progresses rather slowly over a period of a few weeks and then it may recede gradually without treatment or the rabbit may succumb. The epiphora observed in cases that are long standing causes the fur to fall out along the side of the face. Very little experimental work has been carried out on the condition.

Phase I. The body temperatures of four control rabbits and six rabbits affected with conjunctivitis were taken daily for a period of 21 days.

1. The average temperature for the control rabbits was 103.48° F. (39.67° C.).

2. The average temperature for the infected rabbits was 103.78° F. (39.83° C.).

3. The difference between the averages of the control and infected animals was 0.30° F. (0.16° C.).

4. There were no temperature differences noted between the male and female rabbits.

5. Conjunctivitis in the rabbit is not considered a febrile disease.

Phase II. The transmissibility of catarrhal and purulent conjunctivitis was determined by direct contact with eight rab-

bits, four of which were controls and the remaining four were infected with either catarrhal or purulent conjunctivitis.

1. The control animals failed to develop conjunctivitis within the 90 day contact period.

2. The indications are that neither catarrhal nor purulent conjunctivitis are transmissible from one rabbit to another by direct contact.

Phase III. The transmissibility of conjunctivitis was determined by direct swab methods. Two infected rabbits were used for the source of the material that was swabbed on the eyes of four normal rabbits.

1. An initial active hyperemia of the conjunctiva was noticed following swabbing of the eyes. This subsided in 72 hours following the swabbing of the eyes.

2. After the initial active hyperemia subsided, there were no other symptoms of conjunctivitis observed during the 90 day observation period.

3. This phase would indicate that the secretions did not contain the causative agent of conjunctivitis.

Phase IV. The susceptibility of guinea pigs to the purulent exudate from conjunctivitis was studied. Two guinea pigs were inoculated subcutaneously and two were inoculated intraperitoneally. They were observed at daily intervals for 21 days.

1. There were no noticeable symptoms in the guinea pigs injected either subcutaneously or intraperitoneally with the

exudate from the eyes of rabbits with conjunctivitis.

2. This phase would indicate that the guinea pigs examined were not susceptible to rabbit conjunctivitis.

3. The exudate from the conjunctiva of rabbits had no systemic effect on the guinea pigs.

Phase V. The hemogram in conjunctivitis rabbits and normal rabbits was conducted. Five normal rabbits were used as controls and six rabbits of both sexes with conjunctivitis were used for the comparison.

1. The five per cent level of significance was approached with the mean difference of the mean corpuscular volume between the normal and infected animals.

2. A macrocytosis existed to some degree as evidenced by the increase of the mean corpuscular volume of the blood.

3. The erythrocyte counts of the normal rabbits fell in the normal ranges listed by different investigators.

4. The mean difference between the total erythrocyte counts of the control and infected rabbits approached the five per cent level of significance. However, the hemoglobin levels were not decreased in the infected animals, so the decrease in the total erythrocytes of the infected animals did not have too much importance.

5. No apparent anemia existed in either the control animals or the infected animals.

6. The mean difference in sedimentation rate between the infected and control animals was highly significant.

7. The hematocrit values showed no significant mean difference between the two groups.

8. The hemoglobin values did not show any significant difference between the means of control and infected animals.

9. Conjunctivitis did not alter the specific gravity of the blood of the rabbits.

10. The total leukocyte counts of the control and infected animals failed to show any mean significant difference.

Phase VI. The anatomical location of the nasolacrimal duct and canal were determined.

1. The orbital opening of the nasolacrimal canal is on the ventral border of the palpebra tertius, at its junction with the lower eyelid.

2. The osseous opening is at the base of the hamulus lacrimalis.

3. The nasolacrimal duct passes from the corneal surface of the eye to the anterior part of the nares.

Phase VII. A determination of the occlusion of the nasolacrimal duct was carried out by placing aqueous fuchsin in the conjunctival sac and observing the nares for its passage.

1. Epiphora is the result of the occlusion of the nasolacrimal duct.

2. The normal rabbits did not show any occlusion as compared with the abnormal rabbits which showed occlusion.

3. Epiphora continues as long as the nasolacrimal duct is occluded.

Phase VIII. The histopathology of the conjunctiva was studied by using a normal rabbit and a rabbit showing purulent conjunctivitis. The eyes were enucleated with their adnexa and tissue sections were made.

1. Epithelial plaques were present in the sub-epithelium of the conjunctiva of the infected rabbits.

2. Edema and chemosis were present and there was a slight lymphocytic infiltration of the abnormal conjunctiva.

3. The epithelium appeared to be unchanged.

Phase IX. The bacterial flora of the eye was examined in normal rabbits and rabbits with conjunctivitis. Ten normal and ten rabbits demonstrating either catarrhal or purulent conjunctivitis were used. The bacteria were isolated into pure cultures for a determinative study.

1. The following bacteria were found to be present in the eyes of rabbits showing no conjunctivitis:

a. Sarcina lutea
b. Bacillus subtilis
c. Staphylococcus albus
d. Micrococcus epidermidis

2. The above mentioned bacteria have been found in infected rabbits. In addition, Corynebacterium xerose has been isolated.

3. The bacterial flora of the eye is in a dynamic state.

4. It is believed that a possibility of several bacteria being the etiological factor of conjunctivitis in the rabbit.

5. Additional work needs to be conducted in the study of the bacterial flora of the rabbit's eye.